DNA AMPLIFICATION OF DNA USING YFILER

A. SCOPE

AmpF*l*STR Yfiler PCR Amplification Kit (Yfiler) is a Short Tandem Repeat (STR) assay that co-amplifies 17 Y-chromosome STRs in a single PCR reaction. These seventeen short tandem repeat (STR) loci are: DYS456, DYS389I, DYS390, DYS389II (labeled with 6-FAM), DYS458, DYS19, DYS385a/b (labeled with VIC), DYS393, DYS391, DYS439, DYS635, DYS392 (labeled with NED), Y GATA H4, DYS437, DYS438, and DYS448 (labeled with PET).

B. QUALITY CONTROL

B.1 Positive amplification/allelic control (e.g. 007).

This control ensures that the amplification and typing process is working properly. This control is included in the Yfiler typing kit. It is required to run this control with each Yfiler amplification.

B.2 Negative controls:

B.2.1 Reagent Control: This is a tube containing no sample that is carried through the DNA typing process, involving all the reagents used for extraction, quantitation, and amplification. The purpose of this sample is to detect contamination that might occur from the reagents, the environment, or between the evidence samples being processed. At least two reagent controls will be extracted per extraction set, except during the extraction of reference samples where one reagent control may be extracted. All reagent controls will be quantitated, with the reagent control demonstrating the greatest signal being amplified and typed. A reagent control that is amplified and typed shall be amplified utilizing the same primers, instrument model, and concentration conditions as required by the evidence sample with the least amount of DNA; amplified with each amplification kit utilized; and typed using the same instrument model and injection conditions (i.e. injection times and voltage) as the associated evidentiary sample containing the least amount of DNA.

If an evidence sample is re-amplified with the same amplification test kit or system and the template volume is not increased over that of the original reagent control, then re-amplification of the associated reagent blank is not necessary.

B.2.2 Negative amplification control: This control contains only the reagents used to prepare the PCR amplification mixture for each batch of samples, including sample buffer (TE^{-4}). The purpose of this sample is to detect contamination that

might occur from the PCR reagents, the PCR setup environment, or between the PCR reactions being prepared. It is required to run this control with each amplification.

B.3 See DOC ID 12626 regarding processing water controls.

B.4 See DOC ID 1835 to determine reagent expiration dates.

B.5 Protective gloves, a lab coat, and a mask must be worn at all times during plate setup to prevent contamination

B.6 Decontaminate the bench work area with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner.

B.7 All kit components of each new lot of Yfiler and each new lot of LIZ internal size standard must undergo quality control testing prior to being used for the analysis of casework samples.

The 007, 9947A, and an amplification negative control will be amplified using the components of the new lot of Yfiler. The amplified DNA fragments will be separated by electrophoresis on the AB 3130 Genetic Analyzer; typing will be performed using the allelic ladder from the new kit undergoing quality control testing. A new lot of LIZ internal size standard can be used for sizing at this time or the LIZ internal size standard can undergo quality control testing separately since it is not purchased with the other components of the Yfiler kit. The resulting data will be subsequently analyzed using GeneMapper ID-X software. The results obtained from the 007 sample, allelic ladder, and LIZ internal size standard must be as expected and good quality as described in the Yfiler (DOC ID 1776) interpretation guidelines. Multiple volumes (usually 3 and 6 µL) should be amplified to determine the optimum 007 amplification volume. This optimum volume should be noted on the lid of the 007 tube. 9947A must not give any results when 10 µL is amplified. 9947A does not need to be amplified after the initial quality control testing has passed. The negative control must exhibit no alleles. The quality control data will be placed into the critical reagent binder.

B.8 Each new lot of TE^{-4} must undergo quality control testing prior to being used to dilute casework samples; see DOC ID 12626 for more information about this testing.

B.9 Amplification set up must be performed in the pre-amplification room. Amplification plate bases should not be brought into the post amplification room.

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn during plate setup. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn when working outside of a hood.

C.2 All appropriate SDS sheets must be read prior to performing this procedure

C.3 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 Yfiler
- D.1.1 AmpF/STR PCR Reaction Mix
- D.1.2 AmpF/STR Yfiler Primer Set
- D.1.3 AmpliTaq Gold DNA Polymerase
- D.1.4 AmpF/STR Control DNA 007
- D.1.5 AmpF/STR Control DNA 9947A
- D.1.6 AmpF/STR Yfiler Allelic Ladder

D.2 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.3 70% Ethanol (Decontamination)

D.4 TE^{-4} (10mM Tris-HCl- 0.1mM EDTA, 1L)

Add 10 mL 1 M Tris-HCl, pH 8 and 150 µl 0.5 M EDTA to 990mL deionized water. Store at room temperature.

E. EQUIPMENT & SUPPLIES

E.1 Equipment
- E.1.1 Thermalcycler
- E.1.2 Microcentrifuge
- E.1.3 Pipettes
- E.1.4 Vortexer
- E.1.5 96-well plate centrifuge

E.2 Supplies
- E.2.1 Kimwipes
- E.2.2 Sterile aerosol-resistant tips
- E.2.3 Microcentrifuge tubes racks
- E.2.4 AB optical 96-well plates / 0.2 mL tubes
- E.2.5 AB optical strip caps
- E.2.6 96-well plate base

E.2.7 Disposable gloves
E.2.8 Mask
E.2.9 Eye protection (e.g. safety glasses, face shield)
E.2.10 Lab coat
E.2.11 Permanent marker

F. PROCEDURES

F.1 Ensure that the standard curve utilized to determine sample concentration meets the requirements in DOC ID 1785 prior to determining the volume of DNA to amplify. When possible target approximately 0.3 ng-0.5 ng. Fill out an amplification sheet. If 50% or less of a sample has been consumed and the quantity of DNA is less than 0.3 ng, amplification will not be performed; instead, a request to consume the sample will be made. An exception to this can occur if the quantity obtained is very close to the lower end of the target range, e.g. 0.29 ng. In addition, if a sample has been consumed and the total quantity of DNA that can be input into a reaction is less than 0.015 ng, amplification with Yfiler does not have to be performed.

F.2 Dilute samples as needed.

F.3 Determine the number of reactions to be amplified. This should include positive and negative control reactions. Add one or two reactions to this number to compensate for loss during pipetting.

F.4 For each sample add the appropriate quantity of TE^{-4} into each labeled 0.2 mL tube / plate well for a final volume of 10 µL. Add 10 µL TE^{-4} to the amplification negative.

F.5 Prepare a master mix by combining the following volumes of reagents:

Note: *Mix Yfiler reagents by vortexing each tube prior to use.*

# samples	X	9.2 µL PCR Reaction Mix
# samples	X	5.0 µL AmpF*l*STR Yfiler Primer Set
# samples	X	0.8 µL AmpliTaq Gold DNA Polymerase

F.6 Vortex the PCR master mix for 5-10 seconds.

F.7 Dispense 15 µL of the master mix into each 0.2 mL tube / plate well.

F.8 Add the appropriate amount of DNA to each labeled sample tube / plate well. Add the appropriate amount of positive control DNA (labeled 007) to the positive control tube / plate well as determined by the Yfiler kit QC.

F.9 Close tubes or cover wells with plastic strip caps. Place the amplification tubes / plate into a thermal cycler and start the Yfiler method.

F.10 DNA thermal cycling conditions:

Pre-heat:	95°C, 11 minutes
Cycle: (30 cycles)	94°C, 1 minute
	61°C, 1 minute
	72°C, 1 minute
Final extension:	60°C, 80 minutes
Hold temperature:	4-25°C

F.11 After the amplification is complete, remove the tubes / plate from the instrument block and store the amplified products in a refrigerator protected from evaporation, e.g. these tubes and plates can be wrapped in parafilm.

***Note**: The amplified products can be removed from the thermal cycler at any time after reaching 25°C. The amplified products can remain on the thermal cycler overnight or over a weekend at 4°C.*

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 Development and Validation of the AmpF*l*STR Yfiler PCR Amplification Kit: A male specific Single Amplification 17 Y STR Multiplex System, Mulero et al. JFS Vol, 51, No 1, Jan 2006.

H.2 AmpFlSTR Yfiler User's Manual, Applied Biosystems, part number 4358101 Rev C 8/2006.